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Prenatal stress effects on pig development and response to weaning[1]

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ABSTRACT: Exposing a pregnant sow to stress has been shown to affect the resulting offspring. Our objective was to determine if rough handling of pregnant sows altered the physiology of her offspring and if these alterations were different from an experimentally induced model of prenatal stress. Sow treatments consisted of i.v. injections of ACTH (1 IU/kg of BW), exposure to rough handling for 10 min (Rough), or no treatment (Control) once a week during d 42 to 77 of gestation. To determine the plasma cortisol response to treatments, blood (5 mL) was collected from 30 sows after treatment administration. To conduct the prenatal stress study, a separate group of 56 sows was used in 1 of 4 replicates. At birth, production data were collected for each litter, including birth weight, number born, anogenital distance, and pig viability. At weaning, pigs were blocked by BW and sex, and placed in a nursery pen of 6 pigs, with 2 pigs from each treatment group. To assess the effect of treatments on cortisol, corticosteroid-binding globulin (CBG), and hematological cell profiles, blood was collected every other day for 10 d after weaning. Application of treatments caused plasma cortisol concentrations to be greatest in ACTH sows compared with Control sows ($P < 0.001$), with Rough sows having intermediate values ($P = 0.07$). Treatments did not affect the number of pigs born, number of stillborn, or pig viability ($P > 0.40$). The ratio of cortisol to CBG did not differ between treatments ($P = 0.09$). Hematological variables did not differ between treatments ($P > 0.19$). Pigs born to ACTH sows had a smaller anogenital distance compared with controls ($P < 0.03$), with pigs from Rough sows being intermediate. Our data indicate that swine exposed to prenatal stress (ACTH injection) can have alterations in sexual morphology without effects on growth or the immune cell populations measured in this study.

Key words: cortisol, corticosteroid-binding globulin, immune, prenatal, stress, swine

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INTRODUCTION

Prenatal stress, the stress imposed on a pregnant dam that may influence her subsequent offspring, has been shown to have profound effects on behavior and physiology of many species, including monkeys (Clarke and Schneider, 1993), rats (McCormick et al., 1995), guinea pigs (Kapoor and Matthews, 2005), goats (Roussel et al., 2005), humans (Wadhwa, 2005), and swine (Haussmann et al., 2000; Tuchscherer et al., 2002; Kanitz et al., 2003; Otten et al., 2004). Research in our laboratory (Haussmann et al., 2000) has shown that prenatal stress, including restraint and ACTH injection of the sow, caused offspring to have altered neurohormones and adrenal gland morphology, greater plasma cortisol in response to stress, and less ability to heal a wound. Similarly, another research team has recently published a series of papers (Tuchscherer et al., 2002; Kanitz et al., 2003; Otten et al., 2004) in which they used either 5-min restraint stress or exogenous ACTH administration during sow gestation to cause prenatal stress. Those researchers found that prenatal stress impaired the immune function, increased the maximum binding capacity of glucocorticoids receptors in the central nervous system immediately after birth, and caused an increase in fetal cortisol that may be the

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mechanism by which prenatal stress causes its effects. Kranendonk et al. (2005) recently reported that oral administration of glucocorticoids to pregnant sows may prove a useful model for studying its deleterious effects.

The phenomenon of prenatal stress demands our complete understanding to optimize both welfare and productivity in farm animals because prenatal stress can affect both the physiology and behavior of animals and it affects a wide array of species. Thus, to further explore the phenomenon of prenatal stress and its effects in swine, we subjected gestating sows to injections of ACTH or to rough handling during gestation.

MATERIALS AND METHODS

The procedures reported herein were approved by the Purdue University Animal Care Committee.

Animal Procedures

Preliminary Experiment. A study was conducted to assess the plasma cortisol response of sows to treatment using a different group of sows from those that were used in the primary experiment. Second- through fourth-parity sows were subjected to rough handling (n = 10), ACTH (1–39 Corticotropin A, A6303, Sigma Chemical Co., St. Louis, MO) injection (1 IU/kg of BW, n = 11), or served as controls (n = 9) at 60 ± 0.4 d of gestation. Rough handling was conducted as described in the following section for the primary experiment. A baseline blood sample (5 mL) was collected from each sow immediately before treatment. A blood sample (5 mL) was then collected every 30 min for 3 h. To collect blood samples, sows were restrained with a snare and blood samples were collected via jugular venipuncture. Blood samples were collected into 5-mL evacuated tubes containing NaEDTA. All samples were collected within 1 to 2 min of the sow being snared. Blood samples were then immediately placed in an ice bath until they were centrifuged (650 × *g* for 15 min) at 0 to 5°C within 2 h of sampling. The plasma was frozen at –80°C until analysis. These samples were later analyzed for plasma cortisol, as described for the primary experiment.

Primary Experiment: Treatments. All sows (n = 64, parity 2 to 4) were housed in standard gestation stalls (2.21 × 0.61 m). Sows (Landrace × Yorkshire) were either roughly handled (Rough, n = 21) during gestation, given an injection of ACTH (ACTH treatment, 1 IU/kg of BW, n = 21), or left undisturbed (Control, n = 22). All sows were allowed to farrow naturally. All sows were farrowed side-by-side in standard farrowing crates (1.4 × 2.5 m), with metal bars confining the sow within a 0.53- × 2.1-m area in the center of the crate. Pigs had access to the entire pen area. Expanded metal flooring covered the entire crate, except for a 30.5- × 121.9-cm section of one of the side areas that had solid flooring. Each litter received heat from a 250-W lamp suspended above the solid floor area. Sows that tested not pregnant after allocation to treatment or that farrowed fewer than 6 pigs were not used in the study; thus, the final sample sizes were Rough, n = 20; ACTH, n = 16; and Control, n = 20.

To invoke a state of prenatal stress, we roughly handled the Rough sows on d 42, 49, 56, 63, 70, and 77 of gestation. This rough handling consisted of herding the sows down an alley (1.3- × 15.5-m) in the gestation barn for 10 min, with each sow receiving a mild electric shock from a battery-operated livestock prod (Hot Shot, Savage, MN) 3 times at approximately 1, 3, and 7 min. This rough handling regimen was designed to simulate moderate but realistic stress. The second treatment consisted of our ACTH injection model that was developed from previous research (Haussmann et al., 2000). The model of prenatal stress using ACTH injection was developed because, unlike subjecting a sow to a rough-handling stress, injections of ACTH create a more uniform plasma cortisol response among sows. These sows received ACTH on the same day of gestation that the roughly handled sows received their treatment. The control treatment allowed the sows to remain in their gestation stalls undisturbed. The entire study was conducted in 4 replications of n = 16, 18, 10, and 12, respectively, for sows entering the study. Replication 4 consisted of only 2 Control sows and thus their pigs were not used to collect data relative to the stress of weaning because the treatments could not be balanced across the weaning pens.

Production Data Collection

Production data were recorded for these farrowings, including length of gestation, individual birth weight, individual weaning weight, crown-rump length, anogenital distance, and number born. To collect data on anogenital distance, the distances were measured using a cloth measuring tape on the day the pigs were born. Pigs were picked up individually by a handler who held the pigs upside down by their hind legs, while a second person placed the tape to record the distance. The crown-rump length was measured from the base of the head to the base of the tail while the pigs were held in a standing position. The anogenital distance of male pigs, measured as the distance from the anus to the prepuce, was recorded because previous research has shown that prenatal stress altered the masculinity of rats (Keshet and Weinstock, 1995) and the femininity of guinea pigs (Sachser and Kaiser, 1996). The person collecting these data was blind to the treatments. To standardize the anogenital distance for pig size, we created a ratio of crown-rump length divided by anogenital distance. The anogenital distance for females was not measured because previous research in our laboratory (Haussmann et al., 2000) found that because of the very small anogenital distance, enough variation did not exist to determine differences. All pigs were weaned at 19.24 ± 0.15 d of age. An adjusted 21-d weaning weight was calculated (National Swine Improve-

ment Federation, 2003) to account for differences in age at weaning using the formula: adjusted weaning weight = pig BW [2.218 – 0.0811(age) + 0.0011(age^2)].

Tissue Collection and Analysis

At the time of weaning, a litter of pigs was taken from the dam and placed together in a transport box. Blood samples were taken, and 6 pigs (2 from each treatment), blocked by BW and sex, were placed into 1 of 10 weaning pens (n = 60 pigs total). Blood samples (5 mL in an evacuated tube containing K_3EDTA, 8.55 mg per tube) were collected via jugular venipuncture from 1 pig per treatment sow (15 Control, 14 ACTH, and 16 Rough pigs; 22 males and 23 females in total) immediately before weaning and then every other day for 10 d. An aliquot of whole blood (approximately 60 μL) was analyzed using an automated hematology system (QBC Vet Autoread, IDEXX Laboratories Inc., Westbrook, ME) to quantify hematocrit, hemoglobin, mean corpuscular hemoglobin, white blood cells, granulocytes, lymphocyte-monocyte population (the system cannot reliably distinguish these 2 populations, thus they are included as a single population), and reticulocytes. Blood samples were immediately placed in an ice bath until they were centrifuged (650 × *g* for 15 min) at 0 to 5°C within 2 h of sampling. Plasma from these samples was stored at –80°C until later analysis for total cortisol and porcine corticosteroid-binding globulin (**pCBG**).

Cortisol concentrations (nmol/L) were determined for duplicate samples using standard RIA double-antibody kits (DiaSorin Inc., Stillwater, MN). Samples were rerun if the duplicates differed by more than 5%. These kits were previously validated for swine plasma (Daniel et al., 1999). Precision and accuracy of this assay were evaluated in triplicate using a swine plasma pool containing 100 ng/mL of cortisol, resulting in an intraassay CV of 7.1% and an interassay CV of 7.9%. The concentration of pCBG (mg/L) in plasma was measured by a direct ELISA, as described by Roberts et al. (2003). Between assay CV = 13.7% and within-assay CV = 9%. The free cortisol index (**FCI**) was calculated using the ratio of plasma total cortisol to pCBG concentration (le Roux et al., 2002), as validated for swine (Adcock et al., 2006).

Statistical Analysis

Production data at the level of the sow were analyzed using Friedman's test, with replication as the blocking variable. For litter data within sows (anogenital distance, total born, born alive, stillborn, mummies, male:female ratio, birth weight, adjusted weaning weight, and ADG), the data were analyzed using PROC MIXED, with a random intercept (SAS Inst. Inc., Cary, NC). Data for plasma cortisol, which was collected over time, were analyzed using PROC MIXED with an AR(1) serial correlation structure. When significant differences ($P < 0.05$) were detected, Tukey-Kramer or Bonferroni adjustments were used for pairwise comparisons between treatments.

RESULTS

Sow Cortisol Concentrations

Application of treatments to sows at 60 ± 0.4 d of gestation caused sows that received injections of ACTH to have greater plasma cortisol concentrations ($P < 0.001$) than rough-handled sows, which tended to have greater concentrations ($P = 0.07$) than Control sows (Figure 1). Roughly handled sows did show an initial response to the rough handling, which elevated plasma cortisol above baseline ($P = 0.003$). Although this response diminished by 1 h after handling, their plasma cortisol concentrations were maintained above the baseline by approximately 55 nmol/L for the entire sampling duration (Figure 1). Sows that received injections of ACTH exhibited a different plasma cortisol profile, in which peak concentrations were not reached until 1 to 1.5 h after injection. Their peak plasma cortisol concentrations were maintained for a full hour, after which concentrations declined at 2.5 h after ACTH injection (Figure 1). In contrast to the elevation in plasma cortisol of the roughly handled sows, sows receiving ACTH exhibited plasma cortisol concentrations that reached approximately 138 nmol/L greater than their baseline samples and were maintained twice as long. Such differences in treatment responses must be considered carefully when comparing the effects of prenatal stress due to rough handling and ACTH injection on the resultant offspring.

Production Data

Data related to sow productivity and pig preweaning performance can be found in Table 1. Stress and glucocorticoids are known to influence the initiation of parturition; thus, we compared treatments to determine if length of gestation was altered. No differences were found among treatments (Table 1). Stress also has the ability to cause embryonic loss. However, we found no difference ($P = 0.40$) in the total number of pigs born, number of pigs born alive, number of stillborn pigs, ratio of male to female pigs, or the number of mummies (Table 1).

Sows in all treatments produced pigs at birth of similar BW (Table 1), with the SE for litter weight being 0.10, 0.10, and 0.09 kg for the Control, ACTH, and Rough treatments, respectively. Male pigs born to dams that received ACTH had smaller ($P = 0.03$) anogenital distances (a greater ratio of crown-rump length:anogenital distance) compared with pigs from the Rough or Control sows (2.01 ± 0.03, 1.91 ± 0.02, and 1.87 ± 0.03 ratio, respectively; Table 1). A difference in the ratio of 0.1 equals a distance of approximately 0.5 cm. Thus, pigs from sows treated with ACTH had an anogenital

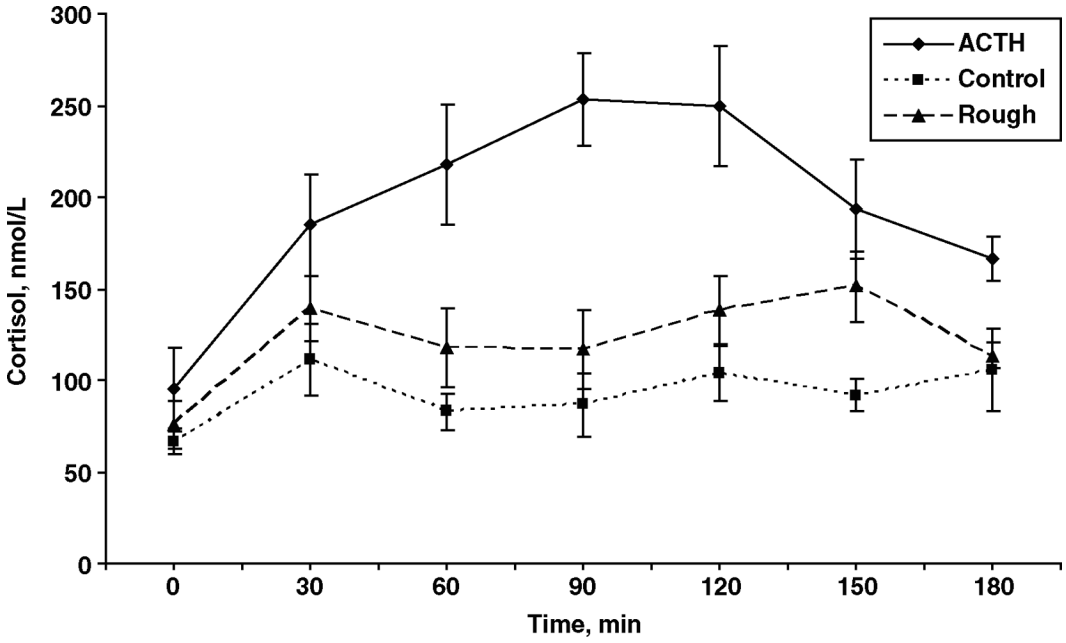

Figure 1. Least squares means (±SE) for the plasma cortisol response of sows from the preliminary experiment that were subjected to ACTH injection (ACTH, 1 IU/kg of BW), served as controls (Control), or were roughly handled (Rough). Blood samples were collected via jugular venipuncture. Sows that received injections of ACTH had greater plasma cortisol concentrations ($P < 0.001$) compared with Rough sows, whose concentrations tended to be greater than concentrations in Control sows ($P = 0.07$).

distance that was 0.75 cm less than pigs from either Control or Rough sows. The ADG from birth to weaning was similar among treatments (Table 1).

Physiologic Response to Weaning

No differences were found among treatments in either plasma cortisol or pCBG ($P > 0.62$) during the 10-d collection period after weaning (data not shown). Total plasma cortisol concentrations were 9.2 ± 0.7, 9.1 ± 0.5, and 9.4 ± 0.8 nmol/L for the ACTH, Rough, and Control pigs, respectively. Mean plasma pCBG concentrations were 1.17 ± 0.08, 1.26 ± 0.12, and 1.41 ± 0.21 mg/L for the ACTH, Rough, and Control treatments, respectively. There was, however, a time effect for both plasma cortisol ($P < 0.001$) and pCBG ($P = 0.001$) concentrations, with plasma cortisol increasing on d 2 in all treatments, whereas pCBG decreased in all treatments on d 2. This inverse response is illustrated by the peak in FCI on d 2 (Figure 2). The FCI did not differ between treatments ($P = 0.09$). Hematocrit and hemoglobin did not differ among the 3 treatments ($P > 0.20$). Hematocrit and hemoglobin exhibited a day effect in which concentrations rose during the weaning period ($P < 0.001$). Lymphocyte-monocyte populations did not differ between treatments ($P = 0.40$).

Table 1. Mean values for production and growth variables

	Treatment			
Item	Control	Rough	ACTH	*P*-value
Gestation length, d	116.00	115.00	116.00	0.64
Total born, no.	9.9	9.0	9.8	0.58
Born alive, no.	8.7	8.2	8.8	0.77
Stillborn, no.	0.9	0.5	0.6	0.44
Mummies, no.	0.3	0.2	0.4	0.51
Birth weight, kg	1.56	1.55	1.49	0.91
Male:female ratio	2.0	1.3	1.3	0.95
CRL/AGD[1]	1.87[a]	1.91[a]	2.01[b]	0.03
Adjusted weaning weight, kg	6.93	6.83	6.45	0.33
ADG, kg	0.26	0.25	0.25	0.40

[a,b]Means within a row with different superscripts differ.
[1]CRL/AGD = the crown-rump length divided by the anogenital distance.

DISCUSSION

The most intriguing finding of this research is that the male pigs of sows treated with ACTH during gestation had a reduced anogenital distance. The reduction in anogenital distance indicates that these pigs were being demasculinized (e.g., Williams et al., 1995, 1998). Measurement of this distance is a simplistic method of quantifying alterations in masculinization that occur during development. In normal fetal development, dihydrotestosterone causes migration of the genital orifice (the prepuce) toward the navel. A lack of this hormone causes no migration and formation of the vulva. Research in rats (Williams et al., 1998) has shown that prenatally stressed male rats have a decreased anogenital distance and express lordosis (Ward, 1972), the fe-

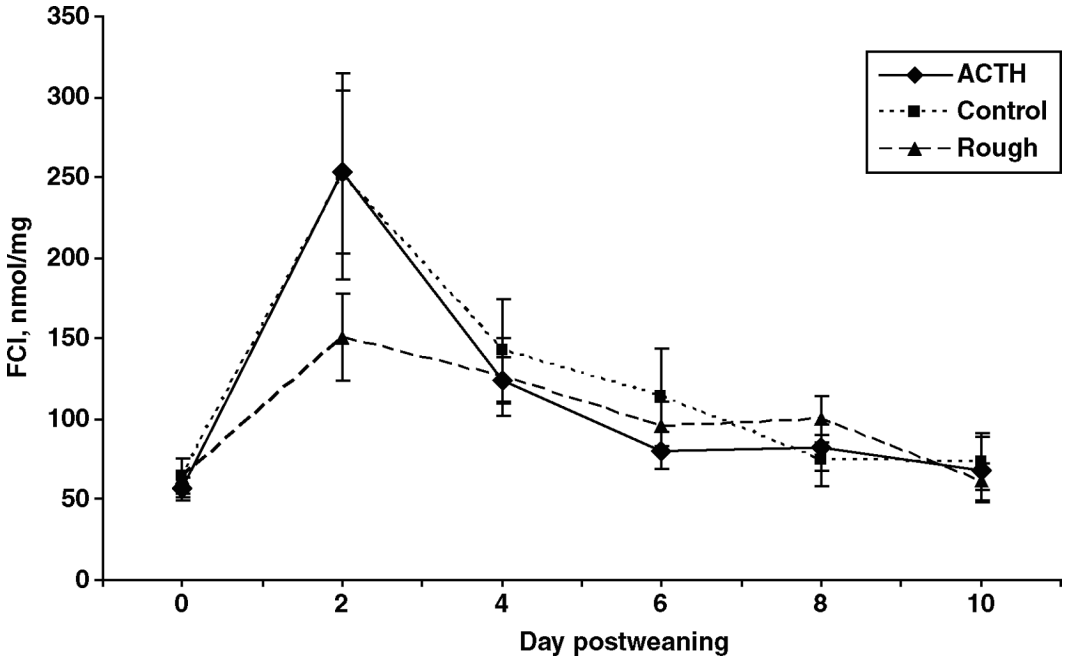

Figure 2. Least squares means (±SE) for the free cortisol index (FCI; nmol/mg) of piglets that were born to sows from the primary experiment that were subjected to ACTH injection (ACTH, 1 IU/kg of BW), served as controls (Control), or were roughly handled (Rough). The FCI was calculated by dividing plasma cortisol concentrations (nmol/L) by porcine corticosteroid-binding globulin concentrations (mg/L) of piglets sampled on d 2, 4, 6, 8, and 10 after weaning (d 0).

male breeding posture. Previous work has shown that the sexually dimorphic area of the preoptic area of the hypothalamus is also demasculinized in male rats (Fleming et al., 1986).

Previous research on rats has found that prenatal stress (or stress hormone administration) can decrease the anogenital distance in male offspring (Keshet and Weinstock, 1995; Williams et al., 1995, 1998) as well as alter their sexual behavior (Ward, 1972). This is the first evidence that such an effect can also occur in swine. However, earlier work by Kattesh et al. (1979) did not find effects of prenatal stress on male development. In their study, sows were subjected to crowding and heat stress during mid-gestation. Data collected from male offspring revealed no effect of prenatal stress on plasma testosterone concentrations in a boar or its libido at approximately 180 d of age. An important difference between these 2 studies was that sows in the research by Kattesh et al. (1979) did not exhibit elevated plasma cortisol concentrations. Thus, the effect on anogenital distances found in the current project could be related to the cortisol response of the sow. The present study did not measure libido or testosterone in boars; thus, it cannot be determined if the reduced anogenital distance was also associated with decreased reproductive function. The current finding of a reduced anogenital distance could have important implications for production agriculture in terms of reproductive ability of boars. Unfortunately, we were not able to follow these pigs into maturity.

The mechanism that causes a reduction in the anogenital distance of swine is unclear. In rats, this reduction has been attributed to maternal glucocorticoids blocking the surge of testosterone on gestational d 18 and 19 (Ward and Weisz, 1980, 1984; Kerchner et al., 1995; Williams et al., 1998). In swine, defeminization of male pigs, especially as related to their behavior, occurs over a much longer period, covering the juvenile period of development (Ford, 1982). Schneider et al. (2004) have shown that ACTH administration to pregnant sows decreases LH concentrations and increases progesterone concentrations in the second trimester. Thus, it is possible that our treatments as early as d 42 of gestation were timed in a manner in which the fetus was susceptible to alteration by maternal hormones.

The majority of prenatal stress research indicates that alterations in prenatally stressed offspring can be attributed to glucocorticoids. However, studies do conflict as to whether the effects of prenatal stress are due to excessive glucocorticoids or opioids. For instance, recent research by Zagron and Weinstock (2006) did not find that male anogenital distance was decreased by excess administration of corticosterone to pregnant rats, although they did find anxiogenic behavior of this treatment, which is associated with prenatal stress. In a rat model of prenatal stress in which male offspring become feminized, Keshet and Weinstock (1995) found that naltrexone given to pregnant rats subjected to stress blocked the feminization of male offspring, sug-

gesting that it is opioids that cause the effects of prenatal stress. Similarly, Reznikov et al. (2005) demonstrated that administration of naltrexone to pregnant rats subjected to restraint stress provided a protective effect on testosterone aromatization of the fetal brain, resulting in the prenatally stressed male offspring not becoming feminized. Other theories exist, including the suggestion by Kaiser and Sachser (2005) that decreased concentrations of dihydroepiandosterone in the pregnant guinea pig may be responsible for causing some of the effects of prenatal stress in this species. Alternative causative agents in the role of altering the fetus during development should be pursued.

The ACTH treatment and the rough-handling treatment did not have the same effect on anogenital distance. Rough handling of livestock involves activation of many physiological and psychological processes. Similarly, the injection of our ACTH treatment not only caused increases in glucocorticoids due to the ACTH, but because the animal was restrained, albeit briefly, it is likely that other stress factors were imposed although not to the extent of rough handling. During a real stress response, as in our rough-handling treatment, activation of the hypothalamic-pituitary axis causes corticotropic releasing factor to stimulate the anterior pituitary gland to produce pro-opiomelanocorticotropin. Further processing of pro-opiomelanocorticotropin produces both β-endorphin and ACTH. Thus, it is possible that treatment differences in this study may be attributed to β-endorphin. Alternatively, a greater magnitude of cortisol increase may be required to cause these differences. A point to consider is that the sows in our primary experiment received treatments 6 times during gestation; therefore, they could have experienced habituation to the treatments, showing less of a cortisol response over time. Although we have shown previously (Lay et al., 1996) that habituation of the cortisol response does not occur after ACTH injection in cattle; the sows may have habituated to the rough-handling treatments and experienced an attenuated plasma cortisol response on successive treatment applications. Indeed, Lay et al. (1996) did find that cattle exhibited a habituated cortisol response to repeated handling and transportation. If sows habituated to rough handling in this study, then the pigs in the ACTH treatment may well have been exposed to much more cortisol than the sows in the Rough treatment. Future work to create an animal model such as the chicken (Lay and Wilson, 2002), which can effectively isolate the multitude of confounding maternal influences observed during prenatal stress, will help to answer these interesting questions as to the role of separate components of the stress response to cause prenatal stress in offspring.

Often characteristic of prenatally stressed offspring is an elevated glucocorticoid response to stress as has been found for rats (Vallee et al., 1997), rhesus monkeys (Clarke et al., 1994), and cattle (Lay et al., 1997a). Indeed, previous research from our laboratory (Haussmann et al., 2000) found that prenatally stressed pigs (ACTH-injected dams) had elevated cortisol concentrations when subjected to mixing stress at 60 d of age, although no differences were detected in plasma cortisol for pigs at 1, 30, or 60 d of age at exsanguination. Other research in swine (Jarvis et al., 2006), which mixed sows together to create prenatal stress, also found greater plasma cortisol in their female offspring when they were themselves mixed as adults. However, Tuchscherer et al. (2002) found no difference in cortisol for controls or prenatally stressed pigs before or after ACTH injection. Also, Kanitz et al. (2003) found lower basal cortisol in prenatally stressed pigs but no difference when they were subjected to ACTH injection.

In the current study, we did not find treatment differences in plasma cortisol concentrations, pCBG concentrations, or the FCI of weanling pigs. These findings are both in contrast and disagreement with previous research. Kranendonk et al. (2006b) found that elevation of glucocorticoids during gestational d 51 to 80 resulted in sows producing pigs that did not differ in their cortisol response to an ACTH challenge compared with controls. In contrast, the pigs from sows that were administered glucocorticoids either before or after this period of gestation exhibited lower plasma cortisol in response to ACTH. Kranendonk et al. (2006b) measured salivary cortisol, which is free cortisol; thus, this measure is functionally comparable with our FCI. In our study, treatments were administered from d 42 to 77 of gestation; a duration similar to that used by Kranendonk et al. (2006b), in which no elevation in salivary cortisol in pigs was noted. Research by Kanitz et al. (2003), using the same model to create prenatal stress as Tuchscherer et al. (2002), revealed lower concentrations of plasma cortisol in prenatally stressed pigs at 3 d of age, with a tendency for lower concentrations at 35 d of age. Kanitz et al. (2003) also found that concentrations of CBG were greater at 3 d of age. Greater concentrations of CBG in conjunction with lower plasma cortisol will dramatically decrease free cortisol. However, subsequent research by Kanitz et al. (2006) indicated a decrease in CBG in prenatally stressed pigs at 1 d of age. Thus to date, there are no consistent responses of plasma cortisol or CBG in prenatally stressed pigs. The peak in FCI 2 d after weaning is indicative of the stress response of the pigs to weaning. A similar increase in salivary cortisol was found in 30-d-old pigs 4 d after mixing with unfamiliar pigs (Koopmans et al., 2006). Maternal deprivation of pigs at neonatal ages ranging from 3 to 31 d caused a transient increase in cortisol (Klemcke and Pond, 1991). In general, glucocorticoids (both endogenous and synthetic) have been shown to have an inhibitory effect on CBG production (Seralini, 1996). A reduction in CBG concentrations can result in an increase in the FCI, especially in the acute stress phase (Bright, 1995) and within a week subsequent to the elimination of the stressor (Heo et al., 2005). The pigs in the current study were approximately 23 d of age when the greater FCI was noted. Changes in measured variables due to age

Table 2. Mean hematological data for pigs in response to weaning

Blood variable[1]	Unit	Treatment			*P*-value
		Control	Rough	ACTH	
Hematocrit	%	25.67	27.75	27.37	0.20
Hemoglobin (Hgb)	g/dL	8.11	8.79	8.62	0.32
Mean corpuscular Hgb	g/dL	31.74	31.68	31.51	0.64
White blood cells	billions/L	11.82	12.07	12.81	0.70
Granulocytes	billions/L	8.17	7.51	8.03	0.95
Granulocytes	%	66.34	69.17	61.73	0.39
Lymphocyte and monocytes (LM)	billions/L	3.72	4.56	4.79	0.40
LM	%	33.60	38.96	38.27	0.19
Platelets	billions/L	511.16	532.54	504.64	0.90
Reticulocytes	%	3.72	3.98	3.84	0.32

[1]Data are presented as the overall means for the 6 blood samples collected during the 10-d postweaning period.

are likely. Comparisons among studies are challenging because they differ in methodology relative to timing of treatments, method of stress inducement, housing of sows and their offspring, as well as genetics.

Why prenatal stress in swine does not consistently increase plasma glucocorticoids of the prenatally stressed pigs is unclear. The majority of the research on prenatal stress has been conducted in rodents. The rate and timing at which physiological systems develop and mature is different between swine and rodents. Rodents are born in an extremely altricial state when compared with swine; therefore, it is likely that prenatal stress is being imposed at different developmental states that will influence the effect it has in changing behavior and physiology of the offspring. Kapoor and Matthews (2005) have shown that application of prenatal stress at different gestational ages in the guinea pig results in different behavioral and physiological responses in their subsequent offspring. Similarly, Jarvis et al. (2006) have shown that stress to the sow during the second trimester compared with the first trimester induces more profound changes in their resultant offspring. Kranendonk et al. (2006a,b) also found that, depending on the stage of gestation (early, middle, late), a variety of changes in both physiology and behavior would occur. Differences between species and differences due to timing of stress during gestation should be expected to result in a different effect of prenatal stress, which could account for the variation in responses of pigs to prenatal stress when compared with rodents.

There was a great deal of data collected during this study that proved not to differ between treatments. This is similar to the findings of Kranendonk et al. (2006b) in which production variables, stress responses, and carcass characteristics were measured, but only a few were different. This observation is just as important as realizing which measures differed. Variables such as gestation length, number born alive, birth weights, adjusted weaning weights, and neutrophil and granulocyte numbers did not differ in the present study. In fact, the means for these nonsignificant data were very close (Tables 1 and 2), indicating that prenatal stress may be inconsequential relative to productivity of swine in agriculture. Thus, although prenatal stress induced by ACTH injection can be a powerful influence on altering the morphologic sex development, in this study it did not alter the measured immune cell populations or sow productivity. These observations indicate that the mechanism by which prenatal stress acts is very specific as opposed to a general deleterious effect on pig physiology. This allows for future research to more precisely identify how stressing a dam affects her fetus.

One consideration that should be entertained is that the effects on offspring attributed to prenatal stress could actually be due to altered maternal behavior or physiology of the sow during the neonatal period of the pig. Research on neonatal stress (e.g., Levine et al., 1958; Denenberg, 1975) has used treatments such as taking the rat pup away from the dam, termed handling, and isolating the pup. In these experiments, this simple neonatal stress typically caused an altered hypothalamic-pituitary axis response in the pups when subjected to subsequent stress. However, Liu et al. (1997) studied neonatal stress and found that when the rat pup is temporarily taken away from its dam, the dam increases her grooming activities toward the pup. This increase in grooming activity was actually found to cause the effects of neonatal stress. We did not measure the behavior of the sow toward her pigs in this study. However, the probability that the treatments the sow received altered her maternal behavior toward her pigs, which caused the effects seen in this study, is unlikely for several reasons. The most compelling reason is that in previous research on prenatal stress in the rat (e.g., Peters, 1990), cattle (Lay et al., 1997b), and swine (Otten et al., 2007) the fetus or newborn has been found to be altered before delivery, effectively ruling out any maternal behavior effect. In addition, Tuchscherer et al. (2002) found that prenatally stressed pigs exhibited a lower proliferative response to both a T-cell mitogen as well as a B-cell mitogen on the first day of life. Similarly, Schwerin et al. (2005) found that prenatally stressed swine (dams were injected with ACTH) pro-

duced fetuses with greater mRNA expression of c-fos in the fetal brain. Another consideration is that the stress treatment to the sow occurred more than 5 wk before parturition. The authors are unaware of any data to indicate that a stressor applied weeks before delivery alters the maternal behavior of the sow. Finally, maternal behavior of rodents is a much more complex, active repertoire than that of a stall-housed sow. Rodents actively groom their offspring and will move them about the cage if necessary; in contrast, a stalled sow has virtually no active contact with her pigs other than snout-to-snout contact along with vocalizations.

Our data indicate that prenatal stress induced by ACTH injections of dams during gestation alters the sexual development of her subsequent male offspring. However, prenatal stress induced by rough handling did not result in negative consequences for the offspring. Although rough handling did not induce a negative consequence, other stressors are likely during gestation that could result in the effects seen from ACTH injections. Some housing environments are known to be stressful and could be creating entire herds of prenatally stressed pigs. In group-housed sows, individuals that reside on the bottom of the hierarchy could be producing litters of prenatally stressed pigs. This project has shown that prenatal stress in swine, induced by ACTH injection which significantly elevates plasma cortisol, is likely caused not by cortisol alone but by other factors as well. Future research needs to develop a model such as the chicken, which will allow more controlled studies on how prenatal stress affects livestock and poultry.

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